Claims

1. A macromolecular conjugate comprising a support to which is coupled at least one targeting molecule in association with an active substance, wherein said targeting molecule is biotin or an analogue thereof possessing binding activity to a biotin receptor.

2. A conjugate of claim 1, wherein the support is a polymer.

3. A conjugate of claim 1, wherein the support is a nanoparticle.

4. A conjugate of claim 1 having the general formula:
$(B\text{-}Q)_n\text{-}P\text{-}(Q'\text{-}A)_m$
wherein B is biotin or a derivative thereof, which is a carrier that binds to a biotin receptor
n, the molar substitution ratio of B in the conjugate, is a number from 1.0 to about 50;
P is a pharmaceutically acceptable linear, branched or dendritic polymer;
A is a pharmaceutically or diagnostic active substance;
m is a number greater than 1.0 to about 1000; and
Q and Q' are independently a covalent bond, or a spacer compound linking biotin, P and A by covalent bonds.

5. The conjugate according to claim 4, wherein at least one of Q and Q' is a spacer compound which contains a biodegradable portion.

6. The conjugate according to claim 5, wherein said biodegradable portion is selected from a disulfide bond, ester linkage, a γ-glutamyl-ε-lysine linkage and a diazo bond, and Gly-Phe-Leu-Gly.

7. The conjugate according to claim 4, wherein n is from 1.0 to about 1.5 and m is from 2 to about 200, more preferably from about 10 to 100.

8. A conjugate according to claim 4, wherein P is a biodegradable polymer.

9. A conjuguate according to claim 8, wherein said biodegradable polymer is selected from a biodegradable carbohydrate polymer or a polymer of amino acids.

10. A conjugate according to claim 4, wherein P is a non-biodegradable polymer.

11. A conjugate according to claim 10, wherein said non-biodegradable polymer comprises biodegradable side chains for covalent linkage to an active substance.

12. A conjugate according to claim 4, wherein said polymer is selected from poly[N-(2-hydroxypropyl)-methacrylamide], dextran or dextran derivatives, chondroitan sulfate, water soluble polyurethanes formed by covalent linkage of PEG with lysine, poly(glutamic acid), poly(hydroxypropyl glutamine), branched chain polypeptides, carboxymethyl cellulose, dendrimers and PEG-dendrimers.

13. A polymer according to claim 12, wherein said polymer is a branched chain polypeptide optionally modified to provide multiple functional groups for coupling of an active substance.

14. A conjugate according to claim 5, wherein said spacer compound Q or Q' has from 1 to 50 atoms in its backbone.

15. A conjugate according to claim 4, wherein said spacer is a diradical spacer comprising optionally substituted alkylene C_{1-50} moiety optionally contained within the chain, double bonds, triple bonds, aryl groups and/or hetero atoms.

16. A conjugate according to claim 15, wherein said spacer compound is derived from disuccinimidyl suberate (DSS), *bis*(sulfosuccinimidyl) suberate (BSS), ethylene glycol*bis*(succinimidylsuccinate) (EGS), ethylene glycol*bis*(sulfosuccinimidylsuccinate) (Sulfo-EGS), p-amino-phenylacetic acid, dithio*bis*(succinimidylpropionate) (DSP), 3,3'-dithio*bis*(sulfosuccinimidylpropionate) (DTSSP), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (Sulfo-DST), *bis*[2-(succinimidyloxycarbonyloxy)-ethylene]sulfone (BSOCOES), *bis*[2-(sulfosuccinimidooxycarbonyloxy)-ethylene]sulfone (Sulfo-BSOCOES), dimethyl adipimidate.2 HCl (DMA), dimethyl pimelimidate.2 HCl (DMP), or dimethyl suberimidate.2 HCl (DMS).

17. A conjugate according to claim 15, wherein said spacer compound is thiol cleavable.

18. A conjugate according to claim 17, wherein said thiol-cleavable spacer is derived from N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), iminothiolane, sulfosuccinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (Sulfo-LC-SPDP), succinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (LC-SPDP), sulfosuccinimidyl 6-[α-methyl-α-(2-pyridyldithio) toluamido]hexanoate (Sulfo-LC-SMPT), 1,4-di[3'-(2'-pyridyldithio)propionamido]butane (DPDPB), 4-succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)-toluene (SMPT) or dimethyl 3,3'dithiobispropionimidate.2 HCl (DTBP).

19. A conjugate according to claim 1, wherein said active substance is a biologically active toxin or a part thereof.

20. A conjugate according to claim 19, wherein said toxin is selected from ricin, abrin, diphtheria toxin, modecin, tetanus toxin, mycotoxins, mellitin, α-amanitin, pokeweed antiviral protein and ribosome-inhibiting proteins, from wheat, barley, corn, rye, gelonin and maytansinoid.

21. A conjugate according to claim 1, wherein said active substance is an alkylating agent selected from chlorambucil, cyclophosphamide, melphalan, cyclopropane; anthracycline antitumor antibiotics such as doxorubicin, daunomycin, adriamycin, mitomycin C, [2-(hydroxymethyl)anthraquinone]; antimetabolites such as methotrexate, dichloromethatrexate: cisplatin, carboplatin, and metallopeptides containing platimun, copper, vanadium, iron, cobalt, gold, cadmium, zinc and nickel, DON, thymidine, pentamethylmelamin, dianhydrogalactitol, 5-Methyl-THF, anguidine, maytansine, neocarzinostatin, chlorozotocin, AZQ, 2'deoxycoformycin, PALA, AD-32, *m*-AMSA and misonidazole.

22. A conjugate according to claim 1, wherein the active substance is an imaging agent.

23. A conjugate according to claim 22, wherein the imaging agent is Rhodamine, fluorescein, Texas red, Acridine Orange, Alexa Fluor (various), Allophycocyanin, 7-aminoactinomycin D, BOBO-1, BODIPY (various), Calcien, Calcium Crimson, Calcium green, Calcium Orange, 6-carboxyrhodamine 6G, Cascade blue, Cascade yellow, DAPI, DiA, DiD, Dil, DiO, DiR, ELF 97, Eosin, ER Tracker Blue-White, EthD-1, Ethidium bromide, Fluo-3, Fluo-4, FM1-43, FM4-64, Fura-2, Fura Red, Hoechst 33258, Hoechst 33342, 7-hydroxy-4-methylcoumarin, Indo-1, JC-1, JC-9, JOE dye, Lissamine rhodamine B, Lucifer Yellow CH, LysoSensor Blue DND-167, LysoSensor Green, LysoSensor Yellow/Blu, Lysotracker Green FM, Magnesium Green, Marina Blue, Mitotracker Green FM, Mitotracker Orange CMTMRos, MitoTracker Red CMXRos, Monobromobimane, NBD amines, NeruoTrace 500/525 green, Nile red, Oregon Green, Pacific Blue. POP-1, Propidium iodide, Rhodamine 110, Rhodamine Red, R-Phycoerythrin, Resorfin, RH414, Rhod-2, Rhodamine Green, Rhodamine 123, ROX dye, Sodium Green, SYTO blue (various), SYTO green (Various), SYTO orange (various), SYTOX blue, SYTOX green, SYTOX orange, Tetramethylrhodamine B, TOT-1, TOT-3, X-rhod-1, YOYO-1 or YOYO-3.

24. A conjugate according to claim 1, wherein the active substance is a radionuclide.

25. A conjugate according to claim 22 wherein the imaging agent is a radionuclide.

26. A conjugate according to claim 22 wherein the imaging agent is linked to a polymer.

27. A conjugate according to claim 22 wherein the imaging agent is incorporated within and/or coated on a surface of a nanoparticle.

28. A conjugate according to claim 4 in which the pharmaceutically acceptable polymer has the sequence of $[(NH_2\text{-}Gly)_4\text{-}Lys_2\text{-}Ser_2\text{-}Lys]_n$-Ala-COOH, where n= 1 to 85.

29. A conjugate according to claim 4 in which the pharmaceutically acceptable polymer has the sequence of $[(NH_2\text{-}X_o)_4\text{-}Lys_2\text{-}Y_2\text{-}Lys]_n\text{-}Z_m$-COOH, where n= 1 to 85; m= 1 to 10; o = 1 to 10; where X is any amino acid, where Y is any amino acid, and where Z is any amino acid.

30. A conjugate according to claim 4 in which the pharmaceutically acceptable polymer has the sequence of $[(NH_2\text{-}Gly)_{16}\text{-}Lys_8\text{-}Lys_4\text{-}His_4\text{-}Glu_4\text{-}Lys_2\text{-}Lys]_n\text{-}Gly_m$-Cys-COOH, where n= 1 to 85; where m = 1 to 10.

31. A conjugate according to claim 4 in which the pharmaceutically acceptable polymer has the sequence of $[(NH_2\text{-}X)_{16}\text{-}Lys_8\text{-}Lys_4\text{-}Y_4\text{-}Z_4\text{-}Lys_2\text{-}Lys]_n\text{-}AA_m$-Cys-COOH, where n= 1 to 85; where m = 1 to 10; where X, Y, Z and AA represent any amino acid independent of each other.

32. A conjugate according to claim 4 wherein P is poly[N-(2-hydroxypropyl)-methacrylamide].

33. A conjugate according to claim 1 or claim 4, wherein the biotin analogue selected from iminobiotin, Biocytin hydrazide, Biotin hydrazide, biocytin, 5-(Biotinamido)pentylamine, Sulfo-NHS(n-Hydroxysuccinimidyl)-Biotin, Sulfo-HNS-hexanyl-biotin (Sulfo-NHS-LD-Biotin), NHS-Biotin, Pentafluorophenyl-biotin,

Pentafluorophenyl-polyethylenoxide-biotin, NHS-biotin Trifluoroacetamide, NHS-Iminobiotin trifluoroacetamide, Maleimido-polyethylenoxide biotin, Maleimido-polyethylenoxide iminobiotin, desthiobiotin, and chloracetyl-biotin.

34. A conjugate according to claim 2, wherein the biotin or biotin analogue is electrostatically or covalently linked to the polymer.

35. A conjugate according to claim 3, wherein the biotin or biotin analogue physically coats a surface of the nanoparticle.

36. A conjugate according to claim 35, wherein the biotin or biotin analogue physically coats the surface of the nanoparticle via electrostatic bonding, hydrogen bonding or hydrophobic bonding.

37. A conjugate according to claim 3, wherein the biotin or biotin analogue is attached to the nanoparticle by covalent bonding.

38. A conjugate according to claim 1, wherein the biotin analogue has cytotoxic or anti-inflammatory activity.

39. A process for synthesising a polymeric conjugate, comprising one or more of the following steps:
a) reacting an active substance with a polymer to form said conjugate;
b) chemically modifying the active substance to provide at least one functional group capable of forming a chemical linkage, and reacting the active substance and polymer to form said conjugate;
c) chemically modifying a target molecule, which is biotin or an analogue thereof, to provide at least one functional group capable of forming a chemical linkage and reacting the target molecule and polymer to form said conjugate;

d) chemically modifying the active substance and the polymer to provide functional groups capable of forming a chemical linkage, and reacting the active substance and polymer to form said conjugate;

e) reacting the active substance with at least one cross-linking agent and reacting the active substance of polymer to form said conjugate;

f) reacting the target molecule with at least one cross-linking agent and reacting the polymer and target molecule to form said conjugate;

g) reacting the active substance and polymer with at least one cross-linking agent and reacting the active substance and polymer to form said conjugate;

h) reacting the active substance directly with a polymeric support to form an intermediate containing one or more molecules of the active substance linked to the polymer, and subsequently coupling the polymer-active substance intermediate to one or more target molecules;

i) coupling one or more target molecules to a polymeric support and subsequently reacting the carrier-polymer intermediate with one or more molecules of the active substance to give a final conjugate containing one or more molecules of the active substance.

40. A conjugate according to claim 1, wherein biotin or an analogue thereof is a first targeting molecule, further comprising one or more second targeting molecules, the second targeting molecules perform a helper function for biotin-binding reactions necessary for uptake and/or transport of biotin in a cell.

41. A conjugate according to claim 1, wherein biotin or an analogue thereof is a first targeting molecule, further comprising one or more second targeting molecules, wherein the second targeting molecules assist in release of the active substance from the conjugate in a cell.

42. A conjugate according to claim 1, wherein biotin or an analogue thereof is a first targeting molecule, further comprising one or more second targeting molecules,

wherein the second targeting molecules promote a biological activity of the active substance.

43. A process for the production of a conjugate having the general formula $(B\text{-}Q)_n\text{-}P\text{-}(Q'\text{-}A)_m$
wherein B, Q, P, Q', A, n and m are as defined in claim 4, said process selected from any one or more of the following steps:
a) reacting A with P to form an intermediate conjugate, and thereafter reacting the intermediate conjugate with biotin;
b) reacting biotin with P to form an intermediate conjugate and therafter reacting the intermediate complex with A;
c) the process of step a) or step b) wherein one or more of biotin, P or A are modified to provide at least one functional group capable of forming a chemical linkage prior to coupling with the other reactants; and
d) reacting one or two of biotin, P or A with Q and/or Q' prior to coupling with the other reactants.

44. A process according to claim 43 wherein Q and/or Q' comprises an optionally substituted alkylene $C_{1\text{-}50}$ moiety optionally within the chain, double bonds, triple bonds, aryl groups, and/or hetero atoms.

45. A process according to claim 43 wherein Q' is a cleavable cross-linking agent containing a disulfide bond.

46. A process according to claim 45 wherein the cross-linking agents are selected from disuccinimidyl suberate (DSS), *bis*(sulfosuccinimidyl) suberate (BSS), ethylene glycol*bis*(succinimidylsuccinate) (EGS), ethylene glycol*bis*(sulfosuccinimidylsuccinate) (Sulfo-EGS), p-amino-phenylacetic acid, dithio*bis*(succinimidylpropionate) (DSP), 3,3'-dithio*bis*(sulfosuccinimidylpropionate) (DTSSP), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (Sulfo-DST), *bis*[2-(succinimidyloxycarbonyloxy)-

ethylene]sulfone (BSOCOES), *bis*[2-(sulfosuccinimidooxycarbonyloxy)-ethylene]sulfone (Sulfo-BSOCOES), dimethyl adipimidate.2 HCl (DMA), dimethyl pimelimidate.2 HCl (DMP), dimethyl suberimidate.2 HCl (DMS).

47. A process according to claim 43 wherein said spacer is selected from disuccinimidyl suberate (DSS), *bis*(sulfosuccinimidyl) suberate (BSS), ethylene glycol*bis*(succinimidylsuccinate) (EGS), ethylene glycol*bis*(sulfosuccinimidylsuccinate) (Sulfo-EGS), p-amino-phenylacetic acid, dithio*bis*(succinimidylpropionate) (DSP), 3,3'-dithio*bis*(sulfosuccinimidylpropionate) (DTSSP), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (Sulfo-DST), *bis*[2-(succinimidyloxycarbonyloxy)-ethylene]sulfone (BSOCOES), *bis*[2-(sulfosuccinimidooxycarbonyloxy)-ethylene]sulfone (Sulfo-BSOCOES), dimethyl adipimidate.2 HCl (DMA), dimethyl pimelimidate.2 HCl (DMP), dimethyl suberimidate.2 HCl (DMS).

48. A process according to claim 43 wherein said spacer is selected from N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), iminothiolane, sulfosuccinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (Sulfo-LC-SPDP), succinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (LC-SPDP), sulfosuccinimidyl 6-[α-methyl-α-(2-pyridyldithio) toluamido]hexanoate (Sulfo-LC-SMPT), 1,4-di[3'-(2'-pyridyldithio)propionamido]butane (DPDPB), 4-succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)-toluene (SMPT), dimethyl 3,3'dithiobispropionimidate.2 HCl (DTBP).

49. A process according to claim 45 wherein the cross-linking agents are selected from N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), iminothiolane, sulfosuccinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (Sulfo-LC-SPDP), succinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (LC-SPDP), sulfosuccinimidyl 6-[α-methyl-α-(2-pyridyldithio) toluamido]hexanoate (Sulfo-LC-SMPT), 1,4-di[3'-(2'-pyridyldithio)propionamido]butane (DPDPB), 4-

succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)-toluene (SMPT), dimethyl 3,3'dithiobispropionimidate.2 HCl (DTBP).

50. A conjugate prepared by a process of claim 43.

51. A conjugate of claim 3, wherein the nanoparticle is prepared by solvent evaporation, complex coacervation, polymer/polymer incompatibility, gelation, interfacial polymerisation or thermal denaturation.

52. A conjugate of claim 3, wherein the nanoparticle is biodegradable.

53. A process for the production of a conjugate of claim 52, which process comprises one or more of the following steps:

a) reacting nanospheres with a targeting molecule to form the conjugate;

b) chemically modifying a targeting molecule to provide at least one functional group capable of forming a chemical linkage and reacting nanospheres and the modified targeting molecules to form the conjugate;

c) reacting nanospheres with at least one cross-linking agent to prepare "activated" nanoparticles which are reacted with a targeting molecule to form the conjugate;

d) reacting a targeting molecule with at least one cross-linking agent and reacting the nanospheres with the reacted targeting molecule to form the conjugate;

e) reacting nanospheres and a targeting molecule with at least one cross-linking agent to the conjugate;

f) reacting nanospheres with at least one cross-linking agent, reacting a targeting molecule with at least one cross-linking agent and reacting the reacted nanospheres and the reacted targeting molecule to form the conjugate; or

g) reacting a targeting molecule with at least one cross-linking agent to prepare an analogue which is reacted with a hydrophobic moiety to form a hydrophobic derivative of the targeting molecule, and then incubating the hydrophobic derivative

of the targeting molecule with a nanosphere in such a manner that the nanosphere is coated hydrophobically with the targeting molecule.

54. A process of claim 53, wherein the cross-linking agent contains a disulfide bond or is cleavable by acid, base or periodate.

55. A process of claim 53, wherein the cross-linking agent is selected from the group consisting of N-(4-azidophenylthio)phthalimide, 4,4'-dithiobisphenylazide, dithiobis(succinimidylpropionate), dimethyl-3,3'-dithiobispropionimidate.2HCl, 3,3'-dithiobis-(sulfosuccinimidylpropionate), ethyl-4-azidophenyl)-1,3'dithiopropionate, sulfosuccinimidyl-2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiobutyrimidate.HCl, N-succinimidyl-(4-azidophenyl)-1,3'dithiopropionate; sulfosuccinimidyl-2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiopropionate, sulfosuccinimidyl-2-(p-azidosalicylamido)-ethyl-1,3'-dithiopropionate, N-succinimidyl-3-(2-pyridylthio)propionate, sulfosuccinimidyl-(4-azidophenyldithio)-propionate, 2-iminothiolane, disuccinimidyl tartrate and bis-[2-(succinimidyloxycarbonyloxy)-ethyl]-sulfone.

56. A process of claim 53, wherein the targeting molecule is cross-linked to the nanosphere or nanoparticle by reaction of the carrier with a carbodiimide and N-hydroxysuccinimide (NHS), and then reacting the NHS derivative with a suitable functional group on the nanosphere.

57. A process of claim 53, wherein the cross-linking agent contains a biodegradable bond.

58. A process of claim 57, wherein the cross-linking agent is cleaved by an esterase, glutathione, or azo-reductase.

59. A conjugate prepared by a process of claim 53.

60. A method for the modification of a polymeric support to introduce functional groups capable of reacting either directly with an active substance or with a chemically-modified form of the active substance, wherein a resulting polymer-active substance intermediate contains one or more molecules of the active substance, said intermediate being suitable for coupling to biotin or an analogue thereof to give a conjugate capable of amplified delivery of the active substance.

61. A pharmaceutical composition which comprises a conjugate according to any one of claims 1 to 21, 24 or 28-38 together with a pharmaceutically acceptable carrier or excipient.

62. A diagnostic imaging composition comprising a conjugate according to any one of claims 22 to 27.

63. A method for the treatment or prophylaxis of disease which comprises administering to a subject a therapeutically effective amount of a conjugate according to any one of claims 1 to 21, 24 or claim 28-38 or a composition of claim 61.

64. A method of claim 63 wherein the disease is cancer.

65. A method of claim 63, wherein the disease is an inflammatory condition.

66. A method of claim 65, wherein the disease is rheumatoid arthritis.

67. A method of claim 65, wherein the disease is Crohn's disease.

68. A method of claim 65, wherein the disease is inflammatory bowel disease.

69. A method of claim 63, wherein the disease is multiple sclerosis.

70. Use of a conjugate according to any one of claims 1 to 21, 24, or 24 to 28 in the manufacture of a medicament.

71. Use of a conjugate according to any one of claims 22 to 27 in the manufacture of a diagnostic imaging agent.

72. A method for the diagnosis of a pathological condition which comprises administering to a subject an effective amount of a conjugate according to claim 22 to 27 or a composition according to claim 62.

73. A conjugate according to claim 4, wherein the linker is biodegradable.

74. A conjugate according to claim 4, wherein the linker is a hydrazone.

75. A conjugate according to claim 4, wherein the linker contains 5-benzoyl-valeric acid.

76. A conjugate according to claim 4, wherein the linker is biodegradable and contains a valine-citrilline dipeptide.

77. A conjugate according to claim 4, wherein the linker is biodegradable and contains a phenylalanine-lysine dipeptide.

78. A conjugate of claim 1, wherein the active substance is a drug selected from platinum derivatives.

79. A conjugate according to claim 78, wherein the platinum derivative is selected from cis-Platin, CarboPlatin, oxaliplatin, multinuclear platinate species including BBR3464 and BBR3005, transdiamminedichloroplatinum (II) (Transplatin), chlorodiethylenetriammineplatinum (II), Platinum IV compounds, spiroplatin, platin-phosphine derivatives.

80. A conjugate of claim 1, wherein the active substance is doxorubicin or an analogue thereof, including daunorubicin, daunomycin, epirubicin, adriamycin.

81. A conjugate of claim 1, wherein the active agent is a cytotoxin selected from anti-folates including methotrexate and dichloromethatrexate.

82. A conjugate of claim 1, wherein the biotin is a hydrazidyl derivative of biotin.

83. A conjugate of claim 1, wherein the biotin is chloracetyl biotin.

84. A conjugate of claim 1, wherein the biotin is desthiobiotin.

85. A conjugate of claim 79, wherein the biotin is desthiobiotin.

86. A conjugate of claim 1, wherein the active substance is a dolastatin derivative.

87. A conjugate of claim 86, wherein the dolastatin derivative is auristatin or monomethylauristatin.

88. A conjugate of claim 4, wherein the linker is a valine-citrilline-aminobenzyl-carbamate derivative.